Atomic Energy Control Board
P.O. Box 1046
Ottawa, Canada
K1P 5S9

Commission de contrôle de l'énergie atomique
C.P. 1046
Ottawa, Canada
K1P 5S9

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RBE OF TRITIUM FOR INDUCTION OF MYELOID LEUKEMIA IN CBA/H MICE

by

D.K. Myers, J.R. Johnson,
et al.
CRNL - AECL

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RELATIVE BIOLOGICAL EFFECTIVENESS OF TRITIUM FOR INDUCTION OF MYELOID LEUKEMIA IN CBA/H MICE

A report by D.K. Myers (Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories) and J.R. Johnson (Battelle Pacific Northwest Laboratories), in collaboration with J.S. Jackson (AECL-CRNL), N.J. Gragtmans (formerly AECL-CRNL), D.H. Percy (University of Guelph), A.R. Jones (formerly AECL-CRNL), D.W. Dunford (AECL-CRNL), H.M. Wyatt (AECL-CRNL), and various support staff.

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ABSTRACT

In order to help resolve uncertainties as to the most appropriate quality factor for tritium beta rays, a large experiment was carried out to measure the relative biological effectiveness (RBE) of tritiated water compared to X rays for the induction of myeloid leukemia in male mice of CBA/H strain. The study was designed to estimate the lifetime incidence of myeloid leukemia in seven groups of about 750 mice each; radiation exposures were approximately 0, 1, 2 and 3 grays both for tritiated water and X rays.

The lifetime incidence of leukemia in these mice increased from 0.13% in the control group to 6-8% in groups exposed to higher radiation doses. The results were fitted to various equations relating leukemia incidence to radiation dose, using both the raw data and data corrected for cumulative animal-days at risk. The calculated RBE values for tritium beta rays compared to X rays ranged from 1.0 ± 0.5 to 1.3 ± 0.3. A best estimate of the RBE for this experiment was about 1.2 ± 0.3. A Q value of 1 would thus appear to be more appropriate than a Q of 2 for tritium beta rays.

RÉSUMÉ

Pour aider à dissiper les incertitudes liées au facteur de qualité le plus approprié aux rayons bêta de tritium, une expérience importante a été entreprise pour mesurer l'efficacité biologique relative de l'eau tritiée, par rapport aux rayons X, pour provoquer la leucémie myéloïde chez des souris de souche CBA/H. L'étude était conçue pour évaluer l'incidence de la leucémie myéloïde sur la longévité de sept groupes d'environ 750 souris chacun. Les doses d'irradiation ont été à peu près de 0, 1, 2 et 3 grays à la fois pour l'eau tritiée et les rayons X.

L'incidence de la leucémie sur la longévité de ces souris est passée de 0,13 pour 100 chez le groupe témoin à 6-8 pour 100 chez les groupes irradiés à des doses plus fortes. On a fait correspondre les résultats à diverses équations reliant l'incidence de la leucémie à la dose d'irradiation en se servant à la fois des données brutes et des données corrigées pour le nombre d'animaux-jours de sensibilité à la dose d'irradiation cumulative. Les valeurs calculées d'efficacité biologique relative des rayons bêta par rapport à celles des rayons X se sont échelonnées de 1,0 ± 0,5 à 1,3 ± 0,3. La meilleure valeur

d'efficacité biologique relative pour cette expérience a été d'environ 1,2 ± 0,3. Une valeur de facteur de qualité *(Q)* de 1 semblerait donc être plus appropriée qu'une valeur 2 de rayons bêta de tritium.

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A. INTRODUCTION

There are a number of uncertainties in the most appropriate value for the quality factor for tritium beta-rays. The ICRP [1] has recommended that a value of 1 could be used for practical purposes in radiation protection, and has used Q = 1 in calculating the annual limit on intake of tritiated water [2]. In Canada, the Federal-Provincial Working Group [3] and the AECB [4] have followed this recommendation.

However, the ICRP [1] has also endorsed an equation relating Q to linear energy transfer (LET), which would suggest that the most appropriate Q for tritium β-rays would be in the region of 2. This relationship was the basis for an early ICRP recommendation [5] that 1.7 be used as Q for tritium. A more recent ICRU publication [6] dealing with possible relations between Q and lineal energy has suggested Q = 2 for tritium β-rays. A value of 2 has apparently been adopted in the USSR [7].

Q is intended to represent an acceptable approximation to the best values of relative biological effectiveness (RBE) of a given radiation for induction of stochastic endpoints (cancers and genetic disorders) at low doses and low dose rates, up to 50 mSv per year to the whole body or 500 mSv per year to a given tissue. The reference radiation which has been used in the past by the ICRP is orthovoltage X-rays or, alternatively, radiation with an LET of less than 3.5 keV per μm [1]. The ICRU has similarly used X-rays as reference radiation [5]. There have been few biological studies in which the RBE of tritium β-rays was compared with X-rays at low dose rate [8]. The only relevant study related to cancer induction was a comparison of the RBE of tritiated water (HTO) and X-rays for acceleration of the appearance of mammary tumors in female Sprague-Dawley rats [9, 10]. The best RBE values for this endpoint were found in that study to be close to 1.

Acceleration of the appearance of mammary tumors in rats could be thought of as the promotion of cancers, rather than the induction of cancers. Possible animal models with which to carry out an induction experiment were reviewed [11]. Following this initial feasibility study [11], the decision was taken to carry out a large scale RBE experiment on induction of acute myeloid leukemia in male mice of the CBA/H strain by HTO and by X-rays delivered at a comparable dose rate. The cost of this five-year project was borne by Atomic Energy of Canada Limited Research Company (45%), Atomic Energy Control Board (27%), Ontario Hydro (24%), Hydro Québec (1.8%) and New Brunswick Electric Power Corporation (1.8%). The design of the study has been described elsewhere [11, 12]. The present report describes the results of this study, which was intended to resolve uncertainties in the most appropriate value of Q for tritium β-rays.

B. METHODS

Forty mice (20 breeding pairs) of the CBA/H strain were obtained from the Harwell MRC laboratories in October 1985. This particular strain, which was not commercially available, had been used by Mole and co-workers [13-16]

to study the induction of myeloid leukemia by X- and γ-rays and appeared to be the most suitable for the purpose of the present study.

The mice were bred randomly until a total of 700 breeding pairs was reached by mid-1986. Blood samples from 10 mice were forwarded to the Institut Armand-Frappier, Laval, Québec and shown to be free of infection by pathogenic viruses or bacteria. Five mice were also sent to Charles River Professional Services, St. Constant, Québec, to verify that their genetic profile was consistent with the CBA strain.

Each of the litters used in this study was identified by birth date, these dates varying from 1986 June 14 to 1986 October 10. The young were weaned at three weeks of age and the female young were euthanized with CO_2. At this time, the young male mice were separated into groups of seven mice from different litters. The tag on each cage was marked with a birthdate; the true birthdate was the date indicated ±1 day depending on the mix of litters. This tag followed each cage of mice throughout the remainder of the study until all mice in the cage had died.

At about three months of age (range 85-110 days), about 126 cages containing seven mice each were sorted into seven different groups for radiation treatment; these treatments will be described below. This procedure was repeated a total of six times over the period from September 1986 to January 1987, in order to obtain a total close to 756 mice in each of the seven treatment groups. The date of treatment and groups was noted on the tag for each cage. In addition, each mouse was identified as to the treatment group by a simple coded ear marking.

Treatments with HTO consisted of single intraperitoneal injections of 0.9, 1.8 or 2.7 x 10^8 Bq of tritium per mouse; based on a preliminary study indicating a half-life of 2.2 days for excretion of HTO in urine, these injections were expected to result in total radiation doses of about 1, 2 and 3 Gy. The weight of the mice was checked at the time of the injection; these weights fell in a range from 90% to 110% of the mean. The amount of tritium injected was not corrected for the weight of individual mice. Following the HTO injection, the mice were held for three weeks in a ventilated rack before being returned to their normal living quarters in the animal facility. Actual HTO doses were estimated from periodic assays of HTO in urine over the first two weeks. Radiation doses were calculated by standard procedures, where the average dose to the cells was assumed to be 0.733 times the dose to body water [3]. Further details are given in Appendix A. The average HTO doses for all mice in groups 1, 2 and 3 were found to be 0.85, 1.86 and 3.04 Gy (Table 1). The standard deviation of the average doses as measured in each of the six repetitions was about 7%. Doses to individual mice based on individual urine samples were not measured in this study but were in a parallel study using the same strain of mice and the same techniques [17]; the standard deviation of the HTO doses to individual mice averaged 9%.

The X-ray source for the first groups of mice was a 300 kVcp Muller X-ray tube operated at 200 kVcp. This X-ray set failed part way through the exposure of the second groups; these mice were discarded and subsequent irradiations were carried out with a 150 kVcp tube equipped with a ISO

filter designed to produce an X-ray spectrum with an average energy of 104 keV. The average LET should be essentially the same for the two X-ray sources used in this study; further details are given in Appendix B.

Plastic shoe-box cages containing the mice were positioned in three wooden racks at distances of approximately three to five metres from the X-ray source (Appendix C), and exposed to these X-rays continuously for approximately 23.5 hours per day over a period of 10 days. Initial dose rates were intended to be 0.24, 0.48 and 0.72 Gy per day. The X-ray dose rate was reduced by 45% every two days in order to parallel the anticipated reduction in dose rate from injected HTO. Acrylic "Lucite" plates of various thicknesses were placed in front of the X-ray tube to achieve the required reductions in dose rate. Energy spectra of the X-rays taken with and without the acrylic plates in place showed little effect of the plates on X-ray energy, and hence on the LET. The reductions in X-ray dose rate were monitored using a calibrated ion chamber which displayed both the instantaneous count rate and the total counts accumulated since the beginning of the irradiation. X-ray doses were measured in each group by TLDs positioned on the back and front of the plastic cages; initial calibrations were done by inserting TLDs subcutaneously in mice in the cages, and relating these doses to the doses measured by TLDs positioned on the outside of the cages. This type of calibration was carried out for both X-ray sources. The measured doses from one cage to another in any given rack usually showed a standard deviation of about 8%; the standard deviation of the average doses as measured in each of the six repetitions was about 9% (see Appendix A). The average X-ray doses for all mice in groups 1, 2 and 3 were 1.06, 1.98 and 2.64 Gy respectively (Table 1).

The X-ray and HTO treated mice were held in different rooms during the exposure period. There were no known differences in the temperature, light or amount of handling that the mice were subjected to with two exceptions. The first was the HTO injections themselves, and the second was the groups of mice randomly selected to be held in metabolic cages (Nalge Metabolism Cages, Fisher Scientific) for urine collection (see Appendix A).

Following the radiation treatments, all mice were held in wire-mesh cages with food (Purina chow) and water ad libitum for the remainder of their life. Of the original 5336 mice, 130 were lost from the experiment due to various accidents (e.g., traumatic injury, escape from the cage), leaving a total of 5206 mice divided more or less equally among the seven groups in this study (Table 1). Mice were checked six days per week throughout the study. Animals found dead in their cage or animals which appeared moribund were subject to postmortem examination; animals which looked unhealthy but did not appear moribund were placed temporarily in a separate cage for further observation. At postmortem, a blood sample was taken whenever possible for blood cell counts and for the preparation of a bloodsmear. Any changes in gross pathology were noted and tissue samples were placed in buffered neutral 10% formalin fixative. For most animals, the tissue samples taken for histological examination included liver, kidneys, adrenals, spleen, heart, lungs, thymus, sternum, vertebrae plus any other tissue in which abnormalities were observed at postmortem. Further details are given in Appendix C. Of the total of 5206 mice in the study, 7.5% were found dead in their cages; blood samples were not available for

these particular animals but vertebrae and sternum bone marrow samples were obtained from all animals in the study.

The characteristics of myeloid leukemia (ML) have been described in detail elsewhere [16, 18-20]. A preliminary diagnosis of ML was made at the Chalk River laboratories based on gross pathology, blood cell counts, white blood cell differentials and blood morphology. In addition, slides prepared from fixed tissue samples from all animals were examined by an experienced pathologist from the Ontario Veterinary College at the University of Guelph. In a few instances where there appeared to be a conflict in the preliminary diagnoses, the data were rechecked at both institutes and agreement on the final diagnosis was reached. Confirmed diagnoses of 277 cases of well-developed ML was made. Two additional cases of early stages of incipient ML, one in group X-2 and one in group HTO-2, were made on the basis of histological examination of the bone marrow. These two cases, in which the ML was not yet obvious in other tissues, were included in the totals given in Table 1. Handwritten information on cause of death for all other mice has been collected but has not yet been analysed.

C. RESULTS

The type of result obtained in this study was generally similar to that reported for CBA/H mice by Mole and co-workers [13-16]. Control animals lived for 767 days on average while irradiated mice lived for about 720 days (Fig. 1 and Table 1). The average age at death was decreased by 50.7 days in the three groups treated with X-rays and by 40.7 days in the three groups treated with HTO. No major importance is attached to these particular numbers for calculating RBE values, since the exact date of death for the majority of animals was usually based on a subjective judgement by animal care personnel as to whether or not the animal should be euthanized because it appeared to be moribund or suffering (see Appendix C). It is, however, worth noting that the average decrease in age at death was similar for animals treated with X-rays or HTO.

Most deaths due to ML occurred between 400 and 800 days of age (Fig. 2 and Table 2), i.e. between 300 and 700 days after exposure to radiation. The average age at death from ML was 80-120 days earlier than that from all other causes (Table 1). Similar results were reported by Major and Mole [13]. It is noteworthy that the average age at death from all other causes, excluding ML, was decreased by the radiation treatments (Table 1). The induction of ML accounted for about 14% of the total decrease in average age at death in the irradiated groups.

There was no obvious shift in average age at ML diagnosis with increase in radiation dose (Fig. 2). These results are again similar to those reported by Mole and co-workers [13, 15]. One minor discrepancy was noted between the results observed at the Chalk River and Harwell laboratories. One case of ML was diagnosed in 747 control mice in the present study (Table 1) while zero cases were observed in over 800 control mice at Harwell [15]. This difference is not statistically significant.

In the preliminary design of the present study, it was assumed that the incidence of ML would increase in direct proportion to the accumulated radiation dose at low dose rate [11, 12]. This assumption is apparently incorrect; the incidence of ML appeared to reach a plateau at about 2-3 Gy (Table 1). In this respect, the dose response curve appears to be similar to that observed with X-rays at a high dose rate of about 0.5 Gy per minute; under the latter conditions, the incidence increases with increasing dose to reach a maximum at about 2-3 Gy and subsequently decreases again at higher doses [15]. The decrease at higher doses is assumed to be due to inactivation of ML precursor cells by the higher radiation doses [15, 21]. Similar results have been observed for induction of ML in RF mice [22] and CBA/Cne mice [23] after exposure at high dose rate.

The raw data on ML incidence were subsequently corrected to cumulative incidence per 10^4 mouse-days at risk [9, 10]. If an animal dies fairly early in the study from some other cause, it is no longer at risk for development of ML at a later date. Correction for early mortality by this method [9, 10] is a standard procedure in most studies on radiation-induced cancer in animals, although it was not applied in the studies on ML at Harwell [13-16].

Both the raw data (Table 1) and the corrected data per 10^4 mouse-days at risk (Table 2) were fitted to a variety of equations relating incidence I to radiation dose D. For example, $I = a + bD$ would represent a simple linear dose-response relationship with no correction for cell inactivation, while $I = a + bD \exp(-cD)$ represents the same relationship with addition of an exponential term for cell inactivation. Other commonly used equations are discussed elsewhere [15, 21]. The possible relationship $I = bD^2 \exp(-cD)$ was also tested since this particular equation was favoured by Mole and co-workers [14, 15] as the best fit to their own data on X-ray-induced ML in the same strain of mice.

The results from the curve fitting program [24] are summarised in Tables 3 and 4. This program fits experimental points to any given mathematical function in a manner such that the sum of the squares of the discrepancies between experimental and predicted points is minimal. The tables show the calculated values of the constants a, b and c together with the standard deviation of b (i.e., Δb) and the residual sum of squares for the differences between the calculated and observed points. The degrees of freedom in curve fitting equal the number of data points available (i.e., 4) minus the number of constants (i.e., 2 or 3) in the assumed equation relating I and D; in general, therefore, the residual sum of squares should be smaller for fits involving three constants than for those involving two constants if both equations were equally appropriate. From this viewpoint, one of the best fits to both the X-ray and the HTO data is provided by the simple relationship:

$$I = bD \exp(-cD)$$

where I is the corrected incidence per 10^4 mouse-days at risk.

The goodness of fit of the experimental data to the equation with a cell inactivation term (Fig. 3) compared to a simple linear relationship with no correction for cell killing (Fig. 4) is illustrated in the attached graphs.

The RBE values (and standard deviations) for induction of ML were calculated from the equation:

$$RBE = \frac{b_2}{b_1} \pm \frac{b_2}{b_1} \left[\left(\frac{\Delta b_2}{b_2}\right)^2 + \left(\frac{\Delta b_1}{b_1}\right)^2 \right]^{1/2}$$

where b_2 and b_1 are the slopes b for induction of ML by HTO and X-rays, respectively, and the Δb values are the associated standard deviations. The ratio b_2/b_1 is chosen to define RBE because this ratio should give the RBE at doses approaching zero, which are relevant for radiation protection. The RBE values calculated for the various dose response relationships were all very similar and covered the range from 1.00 to 1.29 (Tables 2 and 3). The RBE for the best fit to the equations $I = bD \exp(-cD)$ for corrected incidence per 10^4 mouse-days at risk was 1.24 ± 0.31 (Table 3). The RBE for the cell inactivation term in the same equation was 1.0 ± 0.28.

All the above fits were carried out with all four data points for X-rays and for HTO. Linear fits to the first three data points were also carried out (Table 5), omitting the point for the highest dose in each instance since this high dose point would be influenced most by the cell inactivation component. The RBE values were in the region of 1.05 to 1.08 (Table 5) but the fits were not as good as for the relationship which incorporated a cell inactivation term.

D. DISCUSSION AND CONCLUSIONS

Selected RBE values for tritium beta-rays compared to X-rays for the production of various biological endpoints are summarised in Table 6. Other RBE values reported in the same studies range from 0.7 to 1.5 [9, 10, 17 and Tables 3-5]. Although it is impossible to exclude the higher or lover RBE values, the best values are usually in the region of 1.1 to 1.2 (Table 6). The data on induction of ML are undoubtedly the most relevant to assessment of the most appropriate Q value for radiation-induced cancer. As it turned out, all of the RBE values listed in Table 6 are, however, similar. If the assigned Q value for tritium beta rays was taken to be a whole number and the reference radiation was taken to be orthovoltage X-rays [1-6], the present studies and other studies revieved in Table 6 would indicate than $Q = 1$ is more appropriate than $Q = 2$ for tritium beta rays.

The method used to calculate absorbed radiation doses from tritium in the present study with mice is the same as that recommended for calculating doses to humans in Canada (3,4). If there is any systematic bias in this calculation, the bias should be identical in the mice and in humans. Thus the measured RBE of about 1.2 for tritium beta-rays compared to X-rays

should be directly applicable to the tritium doses calculated for humans by the same procedure.

The effects of X-rays at low dose rate ($\leq$ 0.7 Gy d^{-1}) in the present study can be compared with those at high dose rate ($\geq$ 0.5 Gy min^{-1}) in previous studies. Following 1 Gy at high dose rate, the uncorrected incidence of ML in CBA mice appears to be about 10% [15, 21, 23] as compared to about 4.5% following 1 Gy at low dose rate (Table 1 and Fig. 3). This suggests that the decrease in dose rate has reduced the efficacy with which X-rays induce ML. However, the cell inactivation component would not appear to be reduced by a similar factor, the maximum incidence at 2-3 Gy being about 6% at low dose rate (Table 1) as compared to 16-17% at high dose rate [15, 21, 23]. The published data on ML incidence following exposure of CBA mice to X-rays at high dose rate [15, 23] were therefore fitted to the equation $I = bD \exp(-cD)$. The term b for induction of ML was 2.5-3 times greater for X-rays at high dose rate than at low dose rate (Table 7). This decrease at low dose rate is similar to that observed in many studies on other biological endpoints [26]. However, the cell inactivation term c was essentially identical at high and at low dose rate (Table 7). This unusual result suggests that inactivation of the ML progenitor cells by high X-radiation doses might be due to apoptosis. Cell inactivation by radiation can be due either to loss of colony forming ability in dividing cells or to apoptosis before cell division. Loss of colony-forming ability in dividing cells is attributed to damage to the chromosomes in the cell nucleus and is usually highly dependent on the dose rate at high doses of low LET radiation. Cell inactivation by apoptosis, on the other hand, is almost independent of dose rate [27, 28] and may be due to damage to the cell membrane rather than to the cell nucleus [28]. The available data on inactivation of ML progenitor cells are consistent with apoptic cell death.

The present data also provided an opportunity to estimate the RBE for induction of ML in CBA/H mice by fission neutrons [29] as compared to X-rays at low dose rate (Table 7). This RBE appeared to be 6.1 $\pm$ 1.6, a value which is somewhat smaller than the values of 20 to 25 suggested for Q of fission neutrons [6]. The RBE of about 2.3 for cell inactivation by fission neutrons as compared to X-rays (Table 7) is even smaller and similar to the values reported for apoptosis [27, 28].

Mole [14, 15] has suggested that the dose-response relationship for induction of myeloid leukemia by low-LET radiation at high dose rate is best fitted by a relationship of the form $I = bD^2 \exp(-cD)$. The present data are insufficient to prove or disprove this hypothesis, although as noted above a relationship involving bD rather than bD^2 provides a good fit to the results (Fig. 2). The relationship involving bD^2 does not help explain the results of dose protraction [14]. The results obtained with X-rays and tritium beta-rays at low dose rate in the present study are in fact rather similar to the more limited data reported for gamma-rays at low dose rate [14], a result which was ascribed by Mole to some important but unidentified factor in leukemogenesis. The relationship involving bD^2 was not invoked by Mole and Davids [29] to fit the data on ML induction by fission neutrons, even though it does seem to provide as good a fit as does the preferred relationship involving bD (Table 7).

An observed decrease in average life expectancy caused by lov doses of radiation at lov dose rates can normally be ascribed to radiation-induced cancers [30]. Since there was a decrease in average age at death of irradiated mice which did not develop ML in the present study (Table 1), it would be of interest to examine the other causes of death for these mice in more detail.

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Table 1. Groups of mice used for the measurement of tritium RBE

Group	Control	X-1	X-2	X-3	HTO-1	HTO-2	HTO-3
Average radiation dose in Gy	0	1.06	1.98	2.64	0.85	1.86	3.04
[Number of mice lost]	(20)	(18)	(11)	(11)	(36)	(15)	(19)
Number of mice remaining in study	747	734	739	746	732	754	754
Average age at treatment (days)	-	99.9 ±6.7	99.3 ±6.9	99.3 ±6.9	99.6 ±7.0	99.3 ±7.2	99.2 ±7.1
Average age at death (days) for all mice in study	767	720	715	714	737	728	714
Average age at death (days) for all other causes excluding ML	768	724	726	721	742	736	722
Average age at death death (days) for ML only	-	640	557	608	663	612	621
Number of cases of ML	1	33	47	45	43	50	60
Incidence of ML per per 100 mice	0.13	4.50	6.36	6.03	5.87	6.63	7.96
Cumulative incidence of ML per 10^4 mouse-days at risk	0.02	0.72	1.03	0.98	0.92	1.05	1.30

Table 2. Ages at which mice developed ML

Age in days	Group						
	Control	X-ray 1	X-ray 2	X-ray 3	HTO-1	HTO-2	HTO-3
	Deaths from ML in each time interval						
200-299	0	0	2	0	0	0	0
300-399	0	1	3	0	0	2	2
400-499	1	6	10	6	3	7	10
500-599	0	7	14	14	8	16	14
600-699	0	3	13	19	18	12	16
700-799	0	13	3	5	10	10	14
800-899	0	3	2	0	3	2	4
900-999	0	0	0	1	1	1	0
	Cumulative incidence per 10^4 mouse-days at risk						
300	00	00	.07	00	00	00	00
400	00	.05	.23	00	00	.09	.09
500	.03	.24	.51	.20	.10	.30	.37
600	.03	.39	.78	.55	.33	.68	.71
700	.02	.44	1.02	.96	.67	.88	1.03
800	.02	.68	1.02	.99	.84	1.03	1.22
900	.02	.73	1.04	.96	.91	1.04	1.30
1000	.02	.72	1.03	.98	.92	1.05	1.30
1100	.02	.72	1.03	.98	.92	1.05	1.30

Table 3. Results of curve-fitting for uncorrected data on incidence of ML per 100 mice

Assumed Dose Response	Treatment	a	b	c	Δb	Residual Sum of Squares
$a + bD$	X-ray	0.98	2.31	-	0.67	3.59
	HTO	1.83	2.31	-	0.89	8.25
	$RBE = b_2/b_1 = 1.00 \pm 0.48$					
bD	X-ray	-	2.77	-	0.37	4.84
	HTO	-	3.09	-	0.58	13.4
	$RBE = b_2/b_1 = 1.12 \pm 0.26$					
$a + bD \exp(-cD)$	X-ray	0.099	6.53	0.39	1.13	0.16
	HTO	0.33	8.10	0.40	3.15	1.30
	$RBE = b_2/b_1 = 1.24 \pm 0.53$					
$bD \exp(-cD)$	X-ray	-	6.69	0.40	0.69	0.17
	HTO	-	8.66	0.41	1.85	1.41
	$RBE = b_2/b_1 = 1.29 \pm 0.31$					
$a + bD \exp(-cD^2)$	X-ray	0.117	4.71	0.105	0.155	0.011
	HTO	0.64	5.06	0.085	2.33	2.81
	$RBE = b_2/b_1 = 1.07 \pm 0.50$					
$bD \exp(-cD^2)$	X-ray	-	4.83	0.106	0.123	0.025
	HTO	-	5.70	0.089	1.28	3.26
	$RBE = b_2/b_1 = 1.18 \pm 0.27$					
$bD^2 \exp(-cD)$	X-ray	-	11.09	0.97	0.042	0.021
	HTO	-	13.46	0.94	0.67	5.11
	$RBE = b_2/b_1 = 1.21 \pm 0.06$					

Table 4. Results of curve-fitting program for corrected data on incidence of ML per 10^4 mouse-days at risk

Assumed Dose Response	Treatment	a	b	c	Δb	Residual Sum of Squares
a + bD	X-ray	0.153	0.376	-	0.107	0.091
	HTO	0.277	0.380	-	0.136	0.190
	RBE = b_2/b_1 = 1.01 ± 0.46					
bD	X-ray	-	0.445	-	0.058	0.122
	HTO	-	0.498	-	0.087	0.308
	RBE = b_2/b_1 = 1.11 ± 0.24					
a + bD exp (-cD)	X-ray	0.014	1.041	0.385	0.187	0.0045
	HTO	0.053	1.227	0.377	0.507	0.035
	RBE = b_2/b_1 = 1.18 ± 0.53					
bD exp (-cD)	X-ray	-	1.064	0.388	0.112	0.0047
	HTO	-	1.316	0.387	0.294	0.038
	RBE = b_2/b_1 = 1.24 ± 0.31					
a + bD exp ($-cD^2$)	X-ray	0.017	0.756	0.103	0.029	0.00039
	HTO	0.101	0.782	0.079	0.365	0.071
	RBE = b_2/b_1 = 1.03 ± 0.48					
bD exp ($-cD^2$)	X-ray	-	0.773	0.104	0.020	0.00068
	HTO	-	0.883	0.083	0.199	0.082
	RBE = b_2/b_1 = 1.14 ± 0.39					
bD^2 exp (-cD)	X-ray	-	1.76	0.96	0.061	0.00044
	HTO	-	2.02	0.91	1.03	0.133
	RBE = b_2/b_1 = 1.15 ± 0.59					

Table 5. Linear regression fits to the equation $I = a + bD$ for first 3 data points only (0-2 Gy), omitting data at the highest dose of X-rays and of HTO

Treatment	a	b	Δb	Residual Sum of Squares
Uncorrected data on incidence per 100 mice:				
X-ray	0.45	3.16	0.60	0.71
HTO	1.14	3.40	1.72	5.11
	$RBE = b_2/b_1 = 1.08 \pm 0.58$			
Incidence per 10^4 mouse days at risk:				
X-ray	0.070	0.513	0.093	0.017
HTO	0.175	0.540	0.266	0.123
	$RBE = b_2/b_1 = 1.05 \pm 0.55$			

Table 6. Selected RBE values for tritium beta-rays compared to X-rays

Endpoint	RBE ± SD	Reference
Induction of dicentric chromosomes in human lymphocytes *in vitro*	1.13 ± 0.18	[25]
Induction of dicentric and ring chromosomes in CBA/H mouse lymphocytes *in vivo*	1.14 (0.85-1.53)*	[17]
Induction of translocations in CBA/H mouse spermatogonia *in vivo*	1.21 (0.77-1.94)*	[17]
Acceleration of the appearance of mammary tumors in female Sprague-Dawley rats	1.21 ± 0.27	[Fig. 5b in ref. 9]
	1.08 ± 0.15	[Fig. 6b in ref. 9]
Induction of myeloid leukemia in male CBA/H mice	1.24 ± 0.31	[Relationship 4 in Table 4]

* 95% confidence interval for RBE [16].

Table 7. Curve fitting for published data on percent incidence of ML in CBA mice after exposure to X-rays or neutrons

Radiation exposure	b	c	Δb	Residual Sum of Squares	Reference
$I = bD \exp(-cD)$					
Fission neutrons	40.6 (40.5)*	0.94 (1.01)*	9.73 -	78. -	[29]
X-ray, high dose rate	15.8	0.43	3.23	159.	[15]
X-ray, high dose rate	22.0	0.50	4.73	24.	[23]
X-ray, low dose rate	6.7	0.40	0.69	0.17	Table 3
$I = bD^2 \exp(-cD)$					
Fission neutrons	133.	1.93	2.82	43.	[29]
X-ray, high dose rate	19.7 (24.)*	0.82 (0.83)*	4.26 -	103. -	[15]
X-ray, high dose rate	27.9 (30.8)*	0.89 (0.94)*	1.64 -	1. -	[23]
X-ray, low dose rate	11.1	0.97	0.04	0.02	Table 3

* Bracketted values are those given in the original publications, using a different curve fitting program. Unbracketted values were derived from the original data using the CRNL curve fitting program.

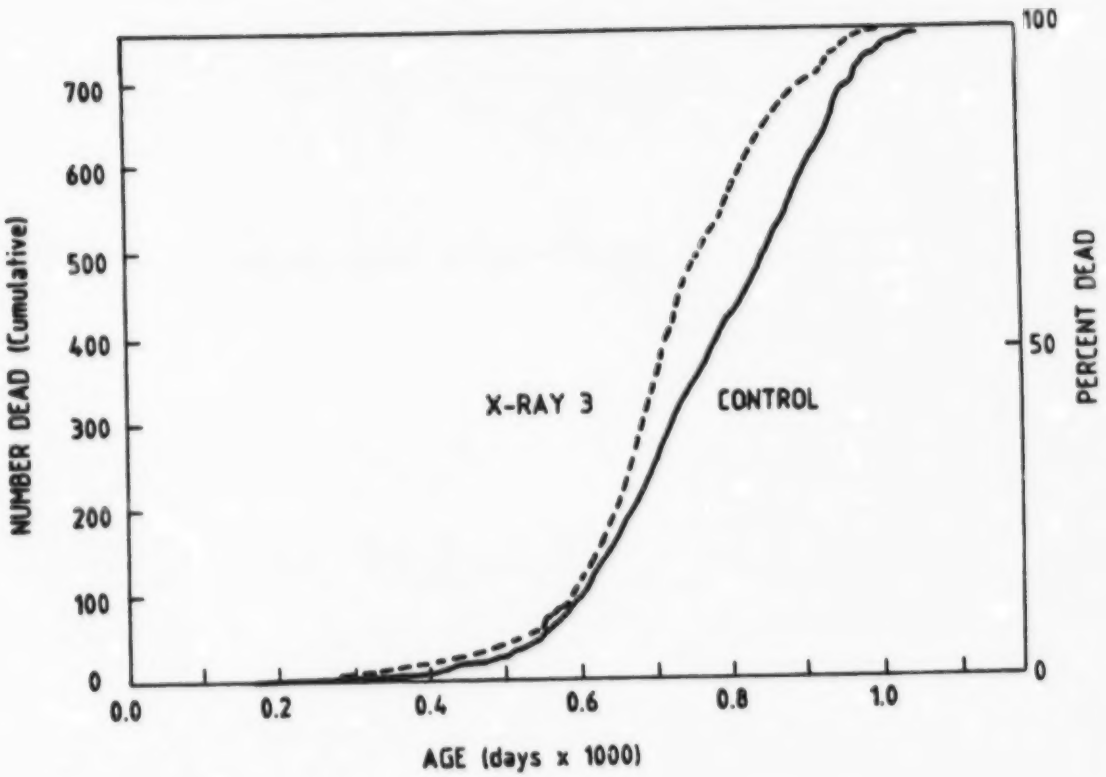


Figure 1. Cumulative mortality of all mice in the control group and in the group designated X-ray 3. For further details on these groups, see Table 1. Cumulative mortality curves for other irradiated groups are not shown but were similar to that for group X-ray 3.

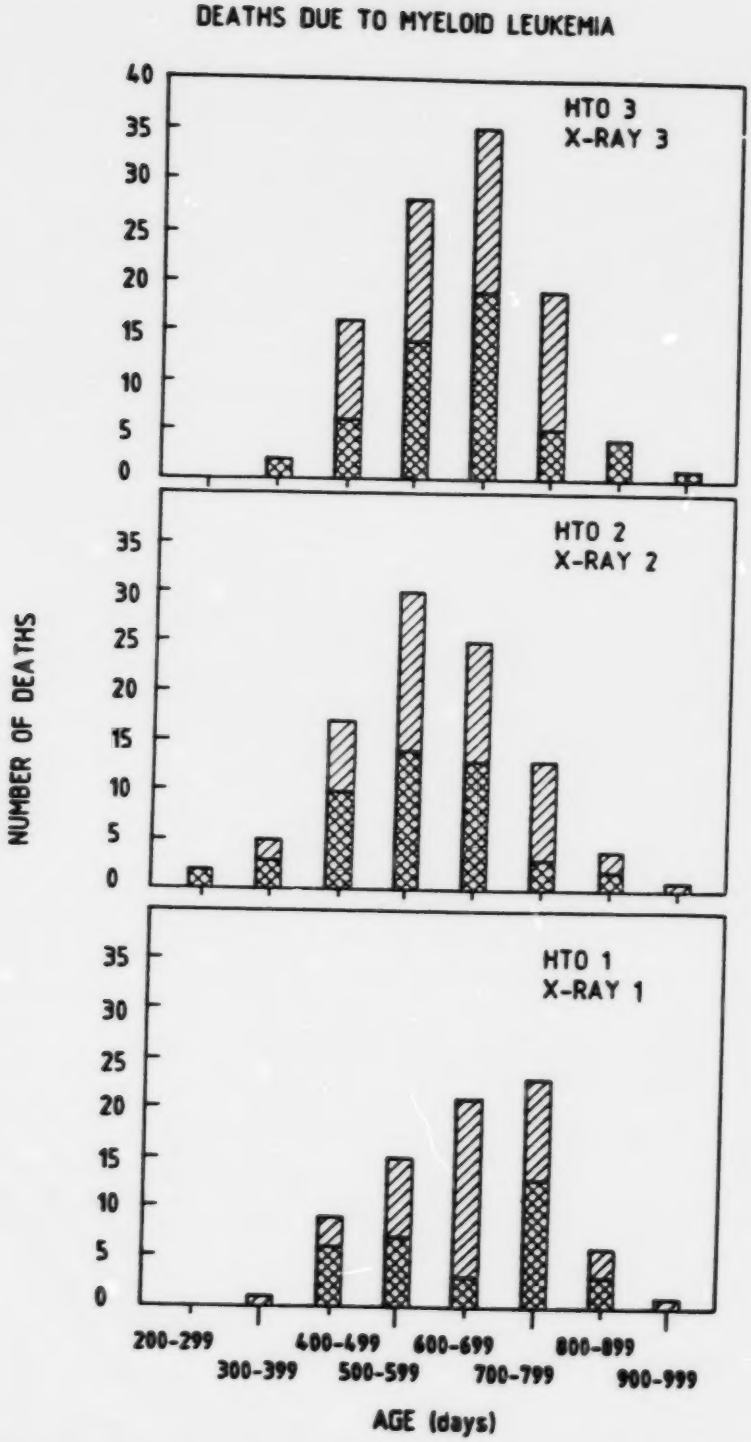


Figure 2. Number of deaths due to ML at 100-day intervals in various groups of irradiated mice. The upper part of each rectangle is for a group treated with HTO and the lover part for a group treated with X-rays. For further details on these groups, see Table 1.

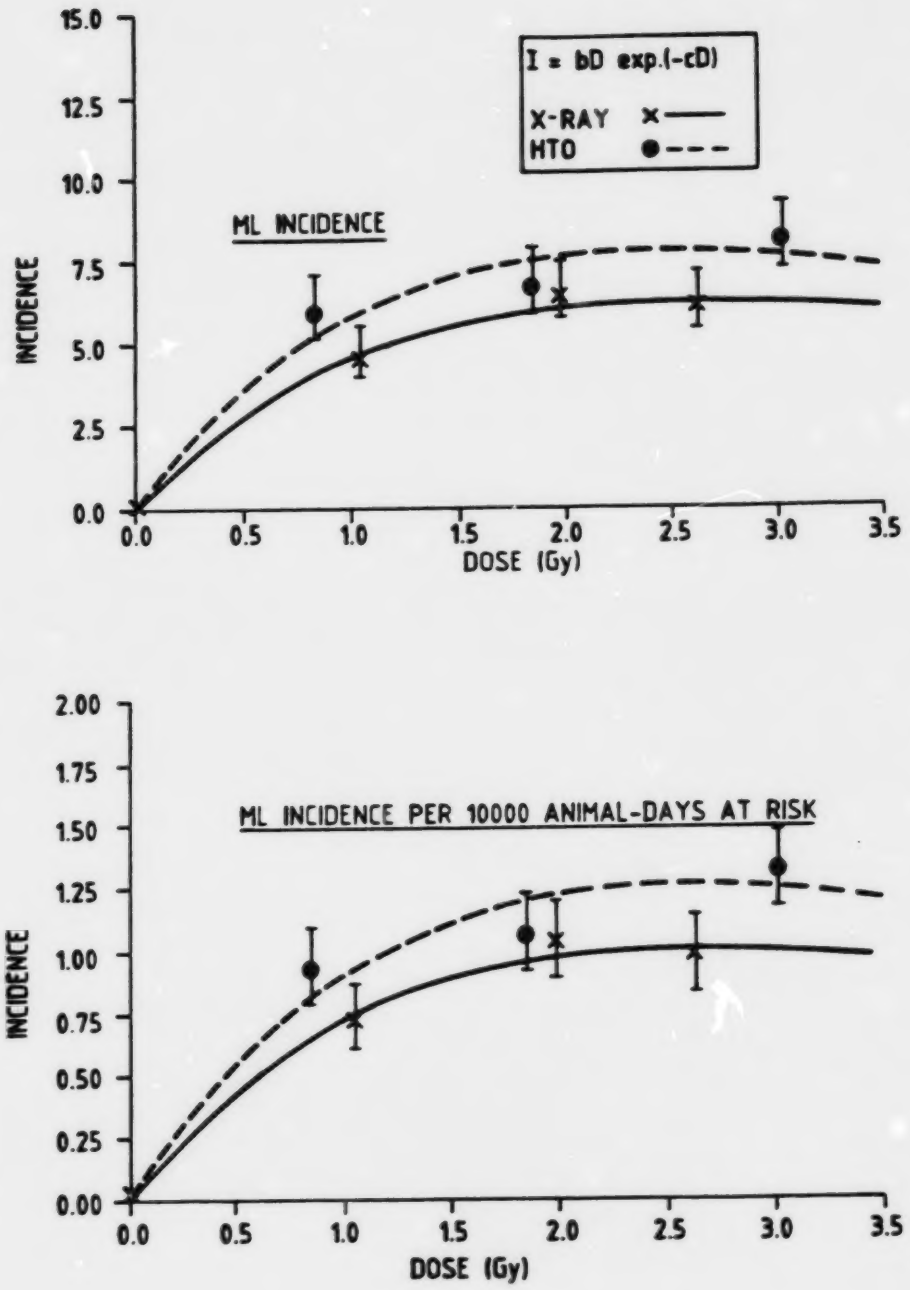


Figure 3. Experimental points and predicted curves for the dose-response relationship I = bD exp (-cD). Top graphs: Uncorrected data for ML incidence per 100 mice (see Table 3). Bottom graphs: Corrected data for ML incidence per 10^4 mouse-days at risk (see Table 4). The vertical lines indicate the standard deviation on each data point.

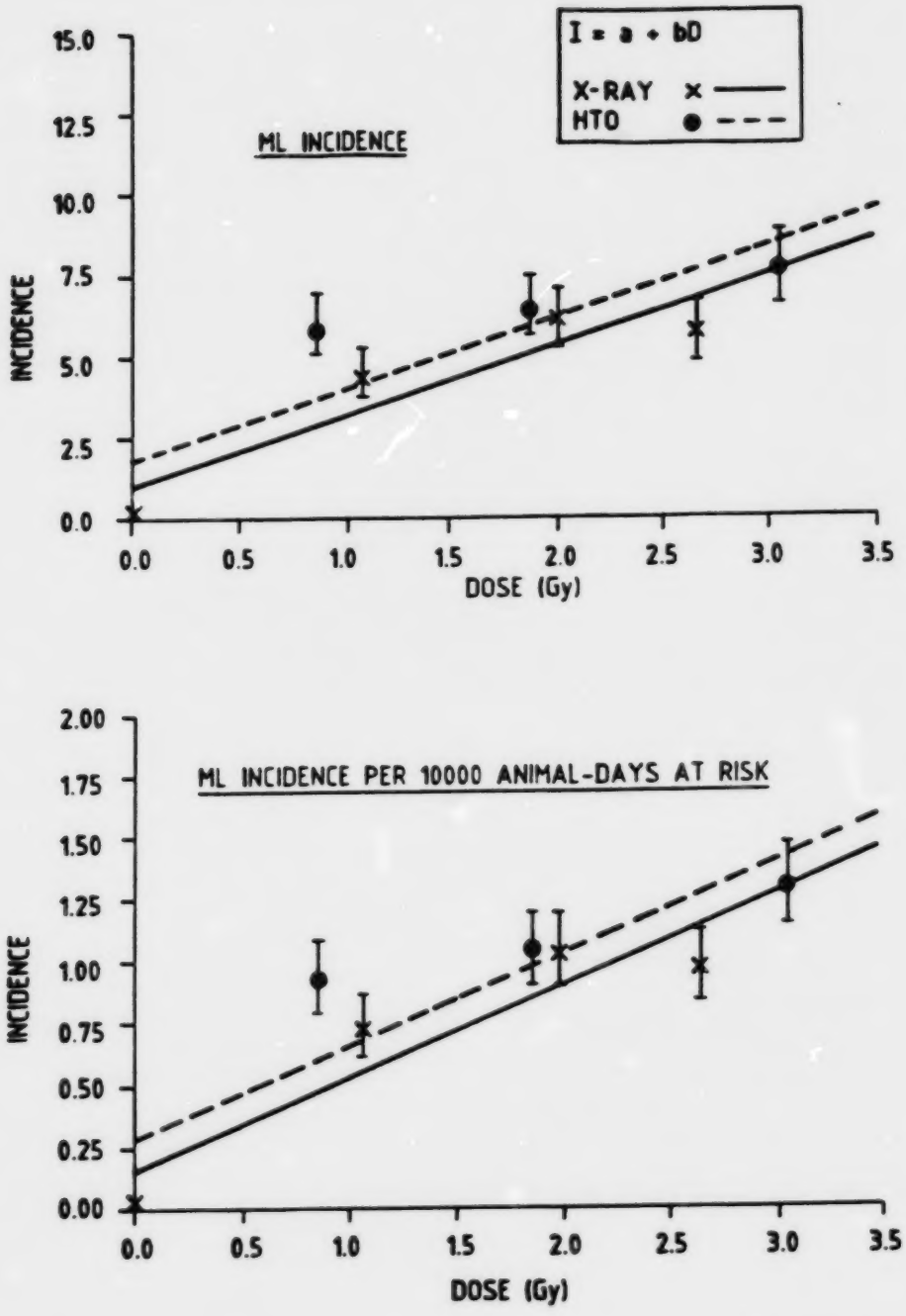


Figure 4. Experimental points and predicted straight lines for the dose-response relationship $I = a + bD$. Top graphs: Uncorrected data for ML incidence per 100 mice (see Table 3). Bottom graphs: Corrected data for ML incidence per 10^4 mouse-days at risk (see Table 4). The vertical lines indicate the standard deviation on each data point.

APPENDIX A

DOSIMETRY FOR THE MYELOID LEUKEMIA TRITIUM RBE STUDY

by

J.R. Johnson

and

A.R. Jones

Dosimetric Research Branch
Chalk River Nuclear Laboratories
Chalk River, Ontario K0J 1J0

Prepared for the Atomic Energy Control Board Panel

1988 February

Introduction

The experimental protocol for this study has been discussed in detail elsewhere (INFO-0175, Rad. Prot. Dosim. 16 161). As stated in these reports, 5000 young male mice were required to carry out the study. The mice for the study were bred at CRNL from stock obtained from Harwell. Their genetic profile has been certified to be consistent with the CBA/H strain by Charles River. This report briefly describes the irradiations and dosimetry used in this study.

The animal facilities at CRNL are not large enough to maintain a mouse colony large enough to produce 5000 male mice all at the same age; nor are the irradiation facilities large enough to irradiate all of the animals at one time. The colony was therefore bred up to a size thought to be capable of producing a set of about 850 male mice every two weeks. This would allow us to do the irradiations in 6 sets, one every two weeks. However, the mice did not breed as fast as anticipated and, in addition, problems with the X-ray set also caused delays, and these combined problems resulted in there being 9 sets of mice irradiated, some by HTO only, some by X-rays only, and some by both.

As mentioned above, the X-ray set failed part way through the irradiation. This necessitated a change from a 300 kVcp set (run at 200 kVcp) to a 150 kVcp set. This change in voltage will result in a change in the photon energy spectrum, which has the potential to change the average LET of the X-rays used in the irradiations. Absorbers were used in order to keep the average LET the same for the two X-ray sets, as explained later.

Tritium Dosimetry

The tritium (as HTO) used in the experiment was obtained from Amersham and calibrated as described in Attachment 1. The working standard mentioned in Attachment 1 is the CRNL standardized HTO that is traceable to an NBS standard. Mice were injected with 0.2 mL, 0.4 mL and 0.6 mL of the HTO in order to deliver a dose of approximately 1, 2 and 3 Gy respectively. Mice were then maintained in cages in a vented rack (Fig. 1) and urine was collected from mice in selected cages (see Table 1) for about 2 weeks post injection. These urine samples were analyzed for tritium concentration by first diluting the samples and then liquid scintillation counting. Standard methods of quench corrections, calibrations, etc. were used. (It should be noted that the method used at CRNL for tritium in urine analyses is cross-calibrated with other Canadian laboratories as part of the inter-comparison program run by Health & Welfare Canada).

The measured tritium concentrations were entered into a computer (LOTUS 1-2-3) as shown in Attachment 2. These data were then fitted to a single exponential of the form:

$$C(t) = A\, e^{-\lambda t}$$

The total dose to body water is then given by:

$$D_{BW} = 51.2 \times 0.0057 \times A/\lambda \text{ rad}$$

and the average dose to cells was assumed to be 0.733 of this quantity (see Rad. Prot. Dosim. 2 245 (1982)). This factor of 0.733 is based on data reviewed in NCRP Report No. 63. Resulting doses are given in Table 2.

X-Ray Dosimetry

Cages containing the mice were positioned in three racks as shown schematically in Fig. 2. Initial calibrations were done by inserting TLD's subcutaneously in mice in the cages, and relating these doses to the doses measured by TLD's positioned on the outside of cages as shown in Fig. 2. (This had to be repeated for the 150 kVcp set.) TLD's at these positions during the actual irradiations were used to assign average doses to sets of mice that are given in Table 3.

The doses delivered to the animals were reduced by approximately one half every two days for a period of 10 days. These reductions were achieved by using an ion chamber which displayed a count rate and accumulated counts. This ion chamber had previously been calibrated against the TLD results on the cages.

Typical results for the control ion chamber are shown below.

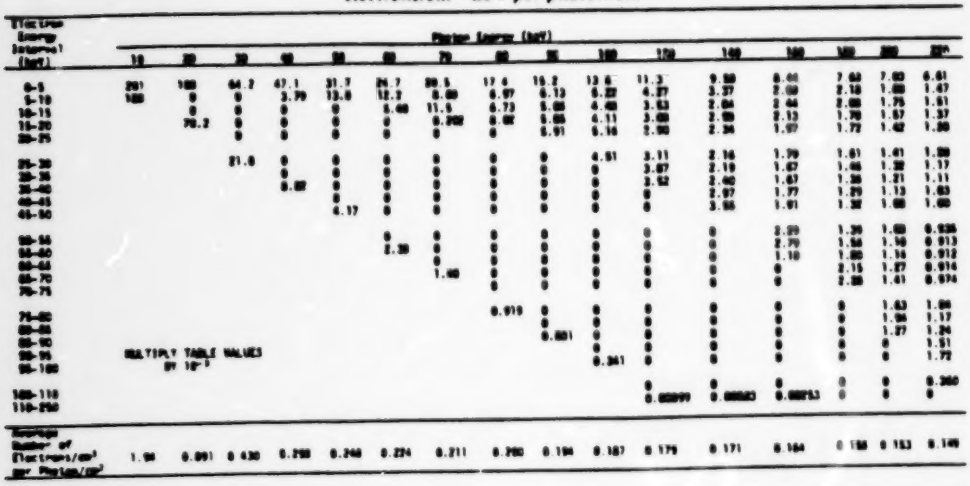
Two-day Period	Count Rate (/s)	Accumulated Counts
First	547	9.44E7
Second	300	5.17E7
Third	164	2.83E7
Fourth	91	1.56E7
Fifth	50	8.77E6

Because the HV X-ray set, which had previously been operated at 200 kV had failed, it was necessary to use a 150 kV machine (also operated at constant potential). To increase the average energy of the spectrum an ISO filter designed to produce a more uniformly monoenergetic spectrum was used. This filter will produce an X-ray spectrum with an average energy of 104 keV, which is approximately equal to the average energy of the spectrum from the 200 kVcp set (see Fig. 3). These spectra will produce primarily electrons in tissue that have essentially equal average energies, and hence the average LET will be essentially the same for the two X-ray potentials used in the study.

The statement that the average energy of the primary electrons produced in tissue from the two different X-ray sets are similar, and that these produce in LET that essentially the same is supported by the work of Todo et al. (Health Physics 43 845-852 (1982)). That report gives the energy spectra

of the primary electrons produced by monoenergetic photons at 10 keV intervals from 10 to 100 keV photon energies, and 20 keV intervals from 100 to 200 keV photon energies. A copy of this data is given below.

Initial electron-energy distribution in water (first-collision spectrum). Number of electrons/cm³ · keV per photon/cm²



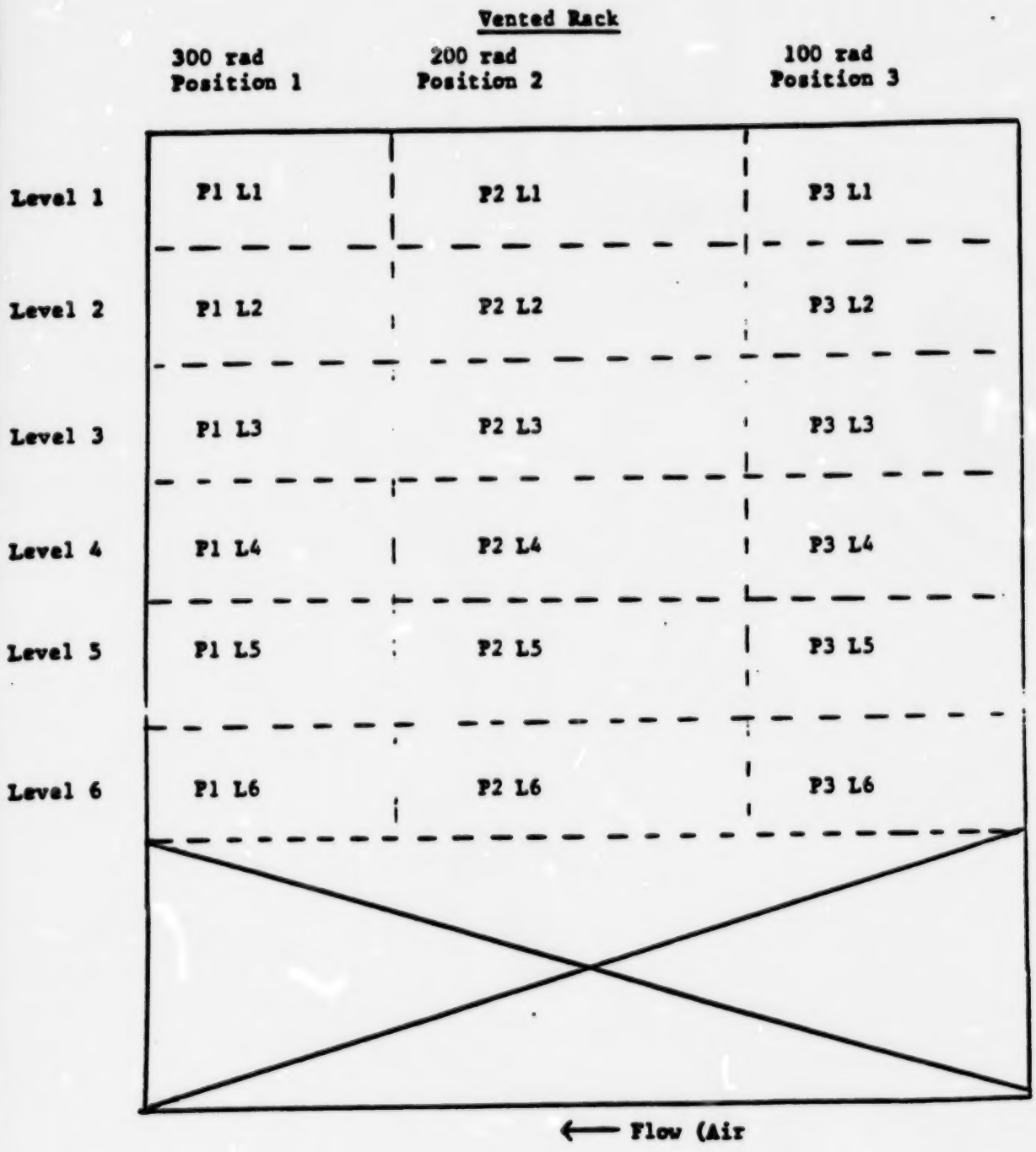
The primary difference in the two X-ray spectra is that the 200 kVp spectra contains photons between 150 and 200 keV that are not in the 150 kVp spectrum, and the intensity of photons between about 40 keV and 60 keV is higher for the 200 kVp spectrum than for the 150 kVp spectrum. In addition the intensity of photons in the 60 to 140 keV range is higher in the 150 kVp spectra than in the 200 kVp spectrum. While a detailed calculation has not been done, it is apparent that the production of primary electrons in energy bands 0 - 5 keV and 5 - 10 keV (which have the most effect on the LET) will be about the same for the two spectra.

To obtain the necessary range of dose rates (11:1) acrylic attenuators of varying thicknesses were needed. Also the arrangement of cages necessitated some difference in the direction of the X-ray beam. Pulse height spectra taken with attenuators varying between 76 and 178 mm and in direction between 0 and 10 degrees did not differ significantly indicating that the average LET would not vary with attenuation or beam direction.

Data Storage

The doses in Tables 2 and 3 are part of the information in the MICE database maintained by the Radiation Biology Branch on an IBM XT. This database also contains the date of birth (± 1 day) and date of irradiation of each mouse in the study. The pathology and histology data are being put into the database as it is accumulated.

FIGURE 1



3 cages at each rack position.

Fig 2

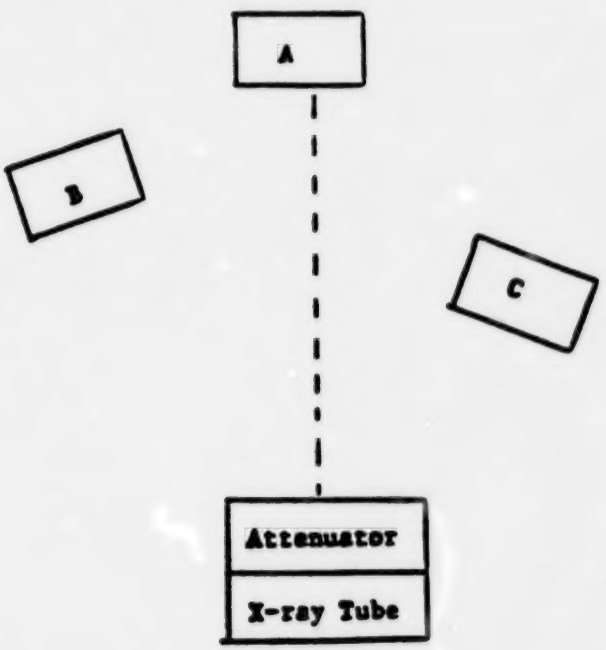
ARRANGEMENT OF CAGES

1*	7
2	8*
3*	9
4*	10*
5*	11
6	12*

*cages with TLDs

Not to scale.

Rack POSITIONS



Not to scale

FIGURE 3: Comparison of the 150 and 200 kVp spectra.

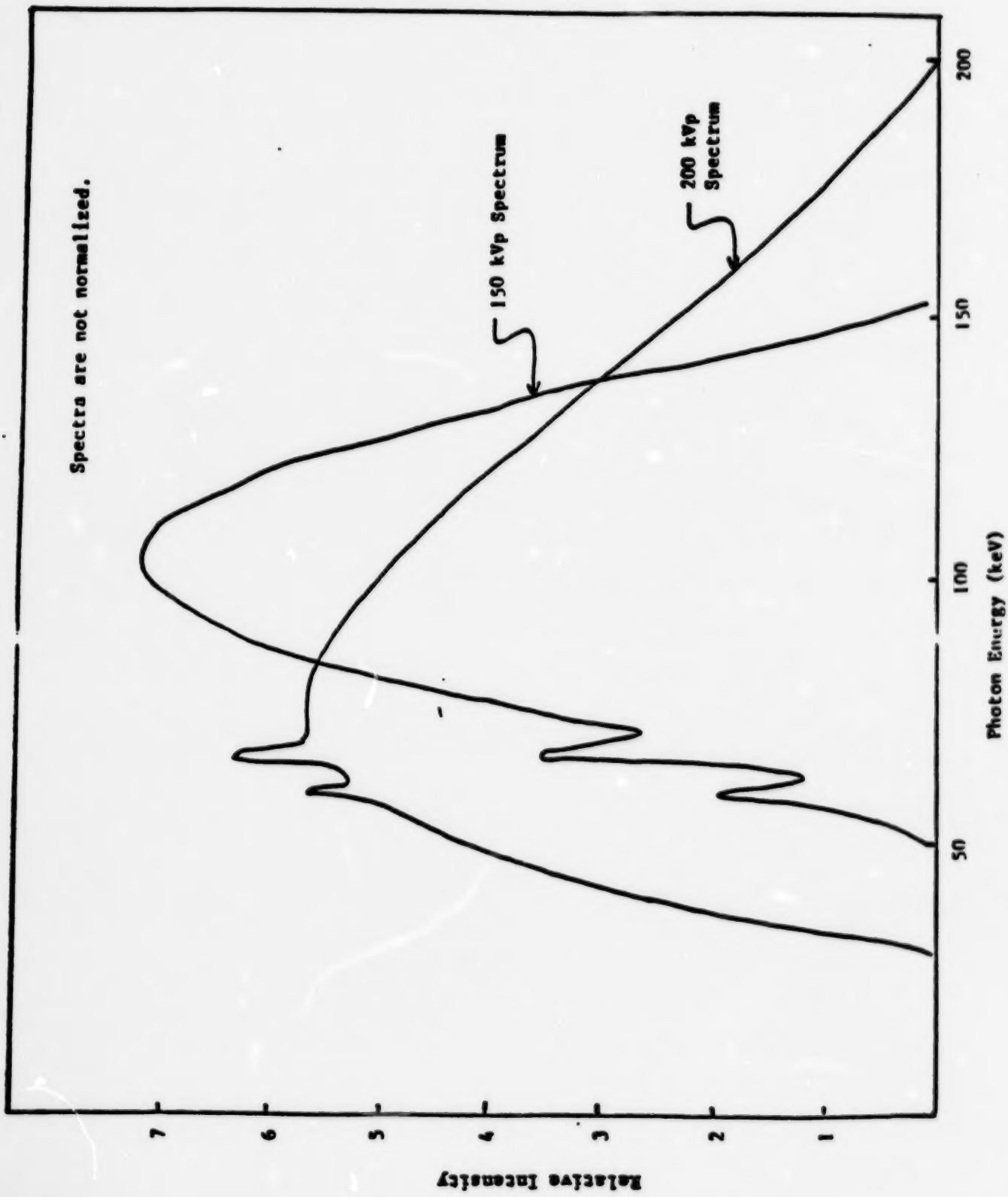


TABLE 1: Typical sampling schedule for tritium in urine.

Day 1	One cage from each of:
	P1L1, P2L1, P3L1, P1L2, P2L2, P3L2
Day 2	P1L3, P1L4, P2L3, P2L4, P3L3, P3L4
Day 3	P1L5, P1L6, P2L5, P2L6, P3L5, P3L6
Day 4	Day 1 schedule
Day 5	Day 2 schedule
Day 8	Day 3 schedule
Day 9	Day 1 schedule
Day 10	Day 2 schedule
Day 11	Day 3 schedule

etc.

TABLE 2: Tritium sets.

Set	Date of Injection	Dose (Gy) HTO1	HTO2	HTO3
1 (1)	86/09/29	0.87	1.99	2.93
2 (2)	86/10/27	0.84	1.81	3.06
3 (3)	86/11/10	0.88	1.70	2.93
3a(4)	86/11/17	0.82	1.64	2.59
6 (5)	86/12/18	0.84	1.91	3.02
8 (6)	87/01/08	0.86	2.11	3.67

TABLE 3: X-ray sets.

Set	Treatment Date (1)	Dose (Gy) XRAY1	XRAY2	XRAY3	Ref. (2)
1	86/09/29	1.04	2.31	3.13	1986/10/15
5	86/11/21	0.92	1.92	2.32	1986/12/17
6	86/12/18	1.15	1.91	2.61	1987/02/23
7	86/12/28	1.05	1.87	2.51	1987/02/23
8	87/01/08	1.02	1.79	2.43	1987/02/23
9	87/01/23	1.20	2.13	2.87	1987/02/23

(1) Date of Start of Treatment
(2) Date of Memorandum from A.R. Jones

ATTACHMENT #1

TRITIUM CALIBRATION 11-9-86

A tritium solution was obtained from Amersham. The nominal amount was 20 Ci in a volume of 4 mL. The solution was standardised in the following way:

0.1365 g diluted to 49.946 g - this is solution A

0.1008 g of A diluted to 49.9086 - this is solution B

0.1013 g of B diluted to 49.6383 - this is solution C

Three aliquots (0.5 mL) of solution C were added to 4 mL of Scintiverse and counted for several cycles.

Three aliquots (0.5 mL) of a working standard (305.01 µCi/L) were added to 4 mL of Scintiverse and counted for several cycles. The quenching in the two sets of samples was found to be the same so that the concentration of C could be obtained directly for the working standard. The data is shown below.

Sample	Average Count Rate	Average	Concentration µCi/L
C1	21198		
C2	21523	21467	50.5
C3	21680		
S1	128847		
S2	129448	129659	305.01
S3	130683		

Therefore:

Solution B = 50.5 X 490.013 = 24745.17 µCi/L
= 9.16 E5 Bq/mL

Solution A = 9.16 E5 X 495.125 = 4.53 E8 Bq/mL

Solution A was made 0.9% NaCl by adding 0.45 g NaCl to the solution.

TRITIUM SOLUTIONS FOR MAIN EXPERIMENT

Three solutions were prepared in a similar manner to that described above. The results are presented below:

Solution A. 0.1629 g of Tritium Soln. diluted to 59.9969 g.
Solution B. 0.1627 g of Tritium Soln. diluted to 59.9964 g.
Solution C. 0.1632 g of Tritium Soln. diluted to 59.9994 g.

The resulting concentrations are, therefore as follows:

Solution A. 4.50 E8 Bq/mL
Solution B. 4.49 E8 Bq/mL
Solution C. 4.51 E8 Bq/mL

ATTACHMENT #2

Sample Data from Set No. 1

Mice set No. 1

Date of injection 86/9/29

Position	Level	Days after injection	Conc. µCi/mL	Ln (conc)	Days after injection	Ave. conc. µCi/mL
p1	11	1	291.00	5.67	1	295.35
p1	12	1	299.70	5.70		
p1	14	2	210.90	5.35	2	213.50
p1	13	2	216.10	5.38		
p1	16	3	149.20	5.01	3	125.35
p1	15	3	101.50	4.62		
p1	11	4	123.00	4.81	4	119.80
p1	12	4	116.60	4.76		
p1	14	7	98.50	4.59	7	88.20
p1	13	7	77.90	4.36		
p1	15	8	27.20	3.30	8	26.90
p1	16	8	26.60	3.28		
p1	11	9	45.00	3.81	9	36.50
p1	12	9	28.00	3.33		
p1	13	10	23.30	3.15	10	32.55
p1	14	10	41.80	3.73		
p1	16	11	32.30	3.48	11	28.15
p1	15	11	24.00	3.18		
p1	12	15	2.89	1.06	15	5.16
p1	11	15	7.43	2.01		
p2	11	1	191.00	5.25	1	171.10
p2	12	1	151.20	5.02		
p2	13	2	134.60	4.90	2	129.25
p2	14	2	123.90	4.82		
p2	16	3	114.90	4.74	3	112.45
p2	15	3	110.00	4.70		
p2	11	4	72.50	4.28	4	72.05
p2	12	4	71.60	4.27		
p2	14	7	54.00	3.99	7	54.55
p2	14	7	55.10	4.01		
p2	15	8	21.40	3.06	8	34.30
p2	16	8	47.20	3.86		
p2	12	9	24.60	3.20	9	21.35
p2	11	9	18.10	2.90		
p2	14	10	13.90	2.63	10	17.85
p2	13	10	21.80	3.08		
p2	15	11	21.40	3.06	11	14.78
p2	16	11	8.16	2.10		
p2	12	15	1.74	0.55	15	2.42
p2	11	15	3.10	1.13		
p3	12	1	91.00	4.51	1	94.15
p3	11	1	97.30	4.58		
p3	14	2	64.60	4.17	2	66.35
p3	13	2	68.10	4.22		
p3	16	3	56.00	4.03	3	52.60
p3	15	3	49.20	3.90		
p3	11	4	33.60	3.52	4	34.10

APPENDIX B

RADIATION QUALITY OF THE 150 kVp AND 200 kVp X-RAYS USED IN THE MYELOID LEUKEMIA TRITIUM RBE EXPERIMENT

(Supplement to the report issued on 1987 February 19.)

by

J.R. Johnson

Dosimetric Research Branch
Chalk River Nuclear Laboratories
Chalk River, Ontario K0J 1J0

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INTRODUCTION

It is common practice to use 200 kVp X-rays as the reference radiation when the effects of radiation of different quality are being compared for the purpose of establishing a quality factor [1,2]. CRNL has used 200 kVp X-rays for past experiments [e.g. 3,4], and intended to in the study of RBE of tritium to produce myeloid leukemia in mice. However, very early in the irradiations, the power supply for the 200 kV X-rays failed, necessitating a change to a 150 kV power supply, with a different X-ray tube with a thin Be window. (In comparison, the 200 kV tube window had a HVL of 0.3 mmCu.) The X-ray spectrum from the 150 kV set would be very soft, and hence the radiation quality would be greater for this tube than for the 200 kV tube. In order that the 150 kV X-rays would have a radiation quality similar to the 200 kV X-rays, an absorber was inserted [5] to "harden" the X-ray spectrum. A comparison of the photon spectra for the two X-ray sets is given in Fig. 1. (It should be noted that both X-ray power supplies were DC.)

It has long been recognized that radiation quality is related to the distribution of ionizing events along the path of a charged particle through tissue. However, there is no consensus [6] on how this distribution can be related mathematically to the RBE. The track averaged linear energy transfer (LET_{∞}) [6] has been used to provide a crude estimate of radiation quality, but this quantity cannot be used to explain differences in RBE for the so-called "low LET" radiations such as tritium beta particles and X-rays [7].

As there is no consensus on which physical quantity best describes radiation quality, it is not very productive to do detailed calculations for the two spectra of Fig. 1 to show that they are very similar in radiation quality. Instead, it was decided to demonstrate that the distribution of electron energies produced in tissue by these two spectra are very similar, and hence their radiation quality must also be very similar.

It is probably important to note in this regard that the effect of window absorption is rarely mentioned for the reference radiation (200 kVp), and different windows can cause variations in the "hardness" of the X-ray spectrum, and hence in the radiation quality.

CALCULATIONAL

Todo et al. [8] have calculated the yield of primary electrons in water (taken to be equivalent to tissue) as a function of photon energy, and their results are given in Table 1. Table 1 also contains the approximate relative number of photons for the 150 and 200 kV X-ray spectra, taken from Fig. 1.

These relative number of photons have been multiplied by the yield per photon from Table 1 to produce the relative number of electrons given in Tables 2 and 3 for the 150 kV and 200 kV X-ray sets respectively. The electrons in each energy range were then summed to give the spectra of primary electrons given in the columns marked (a) in Tables 2 and 3 for the 150 kV and 200 kV X-ray sets respectively. These spectra have been normalized (to 1000 electrons), see columns (b) of Table 4) and plotted on Fig. 2. It will be noted that the 150 kV spectrum (as compared to the 200 kV spectrum) has deficit of electrons below about 15 keV and above 50 keV and an abundance

between about 15 and 50 keV. The overall effect is that the energy weighted average electron energy for the 150 kV electrons is slightly higher than that for the 200 kV electrons. (See Tables 2 and 3.) If the radiation quality can be specified by this quantity, it could be argued that the 200 kV X-rays have a higher quality than the 150 kV X-rays.

It is unlikely that the average primary electron energy is a good measure of radiation quality. These primary electrons will interact to form ionizations and excitations in tissue. In addition, they will produce secondary, tertiary and higher order energetic electrons which in themselves will cause ionizations and excitations. Hence, the radiation quality will be related not only to the energy spectrum of the primary electrons but also to the energy of these "knock on" electrons, or "delta" rays.

Panetzke [9] and Booz and Paretzke [10] have suggested that the Monte Carlo calculations of energy distribution of delta rays produced by primary electrons can be adequately represented by:

$$R(E, E_0) = 1 + \frac{(\frac{1}{2} E_0 - E)^2}{2 E E_0}$$

where E_0 is the primary electron energy, and $R(E, E_0)$ is the number of all electrons set in motion with starting energies above E. This equation was used with the normalized electron spectra of Table 4 to produce the "downward" cumulative distribution of electrons given in Table 5 and Fig. 3.

Fig. 3 shows that the total electron energy spectrum in tissue is essentially equal below about 5 keV. Since, on the scale of biological targets, the distribution of ionization and excitations are essentially equal for electrons above 5 keV, these two spectra should have very similar radiation qualities. If anything, the 200 kV spectrum will have a slightly higher radiation quality than the 150 kV spectrum because of the abundance of electrons between 10 and 30 keV.

CONCLUSION

The radiation quality of the X-rays from the two sets used in the myeloid leukemia tritium RBE experiment will be essentially equal. Differences will be small compared to the differences encountered between 200 kVp sets with different HVL windows.

TABLE 1. Distribution of primary electrons produced in water in the indicated energy range by monoenergetic photons at the indicated energies (Todo 1982). Included at the bottom are the approximate relative number of photons produced by the 150 and 200 kvp X-ray sets.

Electron Energy (keV)	Photon Energy(keV) 10	20	30	40	50	60	70	80	90	100	120	140	160	180	200
0 - 5	201	108	64.2	47.1	31.7	24.7	20.5	17.4	15.2	13.6	11.3	9.6	8.4	7.6	7.0
5 - 10	188	0	0	3.79	13.8	12.2	8.6	7.0	6.1	5.2	4.3	3.4	2.7	2.2	1.8
10 - 15		0	0	0	0	5.4	11.5	6.7	5.1	4.5	3.5	2.8	2.4	2.1	1.8
15 - 20		70.2	0	0	0	0	0.2	8.0	5.9	4.1	3.1	2.6	2.1	1.8	1.6
20 - 25			0	0	0	0	0.0	0.0	5.9	5.2	2.9	2.3	2.0	1.7	1.4
25 - 30			21.8	0	0	0	0.0	0.0	0.0	4.5	3.1	2.2	1.8	1.6	1.4
30 - 35				0	0	0	0.0	0.0	0.0	0.0	3.9	2.2	1.7	1.5	1.3
35 - 40				8.82	0	0	0.0	0.0	0.0	0.0	3.5	2.4	1.7	1.4	1.2
40 - 45					0	0	0.0	0.0	0.0	0.0	0.0	3.0	1.8	1.3	1.1
45 - 50					4.17	0	0.0	0.0	0.0	0.0	0.0	3.6	1.9	1.3	1.1
50 - 55						0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	1.4	1.1
55 - 60						2.38	0.0	0.0	0.0	0.0	0.0	0.0	2.8	1.6	1.1
60 - 65							0.0	0.0	0.0	0.0	0.0	0.0	1.1	1.8	1.1
65 - 70							1.4	0.0	0.0	0.0	0.0	0.0	0.0	2.2	1.3
70 - 75								0.0	0.0	0.0	0.0	0.0	0.0	2.3	1.4
75 - 80								0.9	0.0	0.0	0.0	0.0	0.0	0.0	1.6
80 - 85									0.0	0.0	0.0	0.0	0.0	0.0	1.9
85 - 90	(Multiply all table								0.6	0.0	0.0	0.0	0.0	0.0	1.3
90 - 95	values by 10^{-3})									0.0	0.0	0.0	0.0	0.0	0.0
95 -100										0.3	0.0	0.0	0.0	0.0	0.0
100 -110											0.0	0.0	0.0	0.0	0.0
110 -250											0.00	0.00	0.00	0	0
Average	1.94	0.89	0.43	0.30	0.25	0.22	0.22	0.20	0.19	0.19	0.18	0.17	0.16	0.16	0.15
200kVp Spectrum	0.0	0.0	0.0	1.1	3.3	5.3	5.7	5.5	5.3	5.0	4.0	3.0	1.8	0.8	0.3
150kVp Spectrum	0.0	0.0	0.0	0.0	0.0	1.2	2.5	4.0	6.0	7.4	6.8	3.5	0.8	0.0	0.0

TABLE 2. This table gives the relative number of primary electrons in the indicated energy range produced in water by the photons from the 150 kVp set.

Electron Energy (keV)	Photon Energy (keV)															
	10	20	30	40	50	60	70	80	90	100	120	140	160	180	200	(a)	(b)
0 - 5	0.0	0.0	0.0	0.0	0.0	29.6	51.3	69.6	91.2	101	76.8	33.5	6.8	0.0	0.0	459.45	1148.6
5 - 10	0.0	0.0	0.0	0.0	0.0	14.6	21.5	27.9	36.8	38.6	29.0	11.8	2.2	0.0	0.0	182.41	1368.1
10 - 15	0.0	0.0	0.0	0.0	0.0	6.5	28.8	26.9	30.4	33.2	24.0	9.9	2.0	0.0	0.0	161.55	2019.5
15 - 20	0.0	0.0	0.0	0.0	0.0	0.0	0.5	32.1	35.1	30.4	20.9	9.0	1.7	0.0	0.0	129.77	2271.1
20 - 25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.5	38.2	19.7	8.2	1.6	0.0	0.0	103.13	2320.4
25 - 30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.4	21.1	7.6	1.4	0.0	0.0	63.514	1746.6
30 - 35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.3	7.7	1.3	0.0	0.0	35.317	1147.8
35 - 40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.9	8.4	1.3	0.0	0.0	33.672	1262.7
40 - 45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.4	1.4	0.0	0.0	11.811	502.0
45 - 50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.4	1.5	0.0	0.0	13.953	662.8
50 - 55	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	1.832	96.2
55 - 60	0.0	0.0	0.0	0.0	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	5.088	292.6
60 - 65	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.88	55.0
65 - 70	0.0	0.0	0.0	0.0	0.0	0.0	3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	236.3
70 - 75	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0
75 - 80	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.676	284.9
80 - 85	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0
85 - 90	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.0	3.606	315.5
90 - 95	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0
95 -100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	2.5234	246.0
100 -110	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0
110 -250	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0835	15.0

Energy Weighted average electron energy for the 150kVp X-rays = 13.2 keV.

(a) Sum of the number of primary electrons in the indicated energy range.
(b) Product of column (a) and the midpoint energy of the indicated energy range.

The relative number of photons are those in the indicated interval; that is, those listed under 160 keV are the average in the energy interval 140 to 160 keV. The impact of assuming that all the photons in a range have the energy of the end point of the range is small because the yield of primary electrons in any energy band is relatively independent of energy.

TABLE 3. This table gives the relative number of primary electrons in the indicated energy range produced in water by the photons from the 200 kVp set.

Electron Energy (keV)	Photon Energy (keV) 10	20	30	40	50	60	70	80	90	100	120	140	160	180	200	(a)	(b)
0 - 5	0.0	0.0	0.0	51.8	105	131	117	95.7	80.6	68.0	45.2	28.7	15.2	6.1	2.1	745.8	1864.5
5 - 10	0.0	0.0	0.0	4.2	45.5	64.7	49.0	38.3	32.5	26.1	17.1	10.1	4.8	1.7	0.5	294.6	2209.7
10 - 15	0.0	0.0	0.0	0.0	0.0	28.6	65.6	37.0	26.8	22.4	14.1	8.5	4.4	1.7	0.5	209.6	2620.3
15 - 20	0.0	0.0	0.0	0.0	0.0	0.0	1.2	44.1	31.0	20.6	12.3	7.7	3.8	1.4	0.5	122.6	2145.6
20 - 25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	31.3	25.8	11.6	7.0	3.5	1.4	0.4	81.1	1824.5
25 - 30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.6	12.4	6.5	3.2	1.3	0.4	46.4	1276.1
30 - 35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.5	6.6	3.0	1.2	0.4	26.6	865.2
35 - 40	0.0	0.0	0.0	9.7	0.0	0.0	0.0	0.0	0.0	0.0	14.1	7.2	3.0	1.1	0.4	35.4	1329.0
40 - 45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.9	3.2	1.0	0.3	13.5	572.3
45 - 50	0.0	0.0	0.0	0.0	13.8	0.0	0.0	0.0	0.0	0.0	0.0	10.7	3.4	1.1	0.3	29.2	1388.4
50 - 55	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1	1.1	0.3	5.6	291.8
55 - 60	0.0	0.0	0.0	0.0	0.0	12.6	0.0	0.0	0.0	0.0	0.0	0.0	5.0	1.2	0.3	19.2	1104.8
60 - 65	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.4	0.3	3.8	235.1
65 - 70	0.0	0.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.4	10.1	680.5
70 - 75	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.4	2.2	162.9
75 - 80	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.5	5.5	429.6
80 - 85	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.6	48.0
85 - 90	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0	0.0	0.4	3.6	312.1
90 - 95	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
95 -100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	1.7	166.2
100 -110	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110 -250	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	10.4

Energy Weighted average electron energy for the 200kVp X-rays = 11.8 keV.

(a) Sum of the number of primary electrons in the indicated energy range.
(b) Product of column (a) and the midpoint energy of the indicated energy range.

TABLE 4. Primary electron spectra (a) and normalized primary spectra (b) for the 150 kVp and 200 kVp X-rays. (See Fig. 2.)

Electron Energy (keV)	150 kVp Electrons		200 kVp Electrons	
	(a)	(b)	(a)	(b)
0 - 5	459.5	377.9	745.8	450.0
5 - 10	182.4	150.0	294.6	177.8
10 - 15	161.6	132.9	209.6	126.5
15 - 20	129.8	106.7	122.6	74.0
20 - 25	103.1	84.8	81.1	48.9
25 - 30	63.5	52.2	46.4	28.0
30 - 35	35.3	29.0	26.6	16.1
35 - 40	33.7	27.7	35.4	21.4
40 - 45	11.8	9.7	13.5	8.1
45 - 50	14.0	11.5	29.2	17.6
50 - 55	1.8	1.5	5.6	3.4
55 - 60	5.1	4.2	19.2	11.6
60 - 65	0.9	0.7	3.8	2.3
65 - 70	3.5	2.9	10.1	6.1
70 - 75	0.0	0.0	2.2	1.4
75 - 80	3.7	3.0	5.5	3.3
80 - 85	0.0	0.0	0.6	0.4
85 - 90	3.6	3.0	3.6	2.2
90 - 95	0.0	0.0	0.0	0.0
95 -100	2.5	2.1	1.7	1.0
100 -110	0.0	0.0	0.0	0.0
110 -250	0.1	0.1	0.1	0.0
	1216	1000	1657	1000

TABLE 5. Number of electrons (primary, secondary, tertiary and higher order) above the indicated energy generated from the normalized spectra of Table 4. (See Fig. 3.)

Electron Energy (keV)	150 kVp Electrons	200 kVp Electrons
2.5	1732.5	1763.1
7.5	663.3	593.4
12.5	484.7	386.6
17.5	346.9	252.6
22.5	237.7	175.5
27.5	151.3	125.5
32.5	98.4	97.6
37.5	67.9	80.8
42.5	39.9	59.8
47.5	29.4	50.7
52.5	18.1	33.3
57.5	16.4	29.2
62.5	12.3	17.6
67.5	11.4	14.9
72.5	8.7	10.3
77.5	8.5	7.2
82.5	5.6	4.3
87.5	5.3	3.2
92.5	2.3	1.1
97.5	2.1	1.0

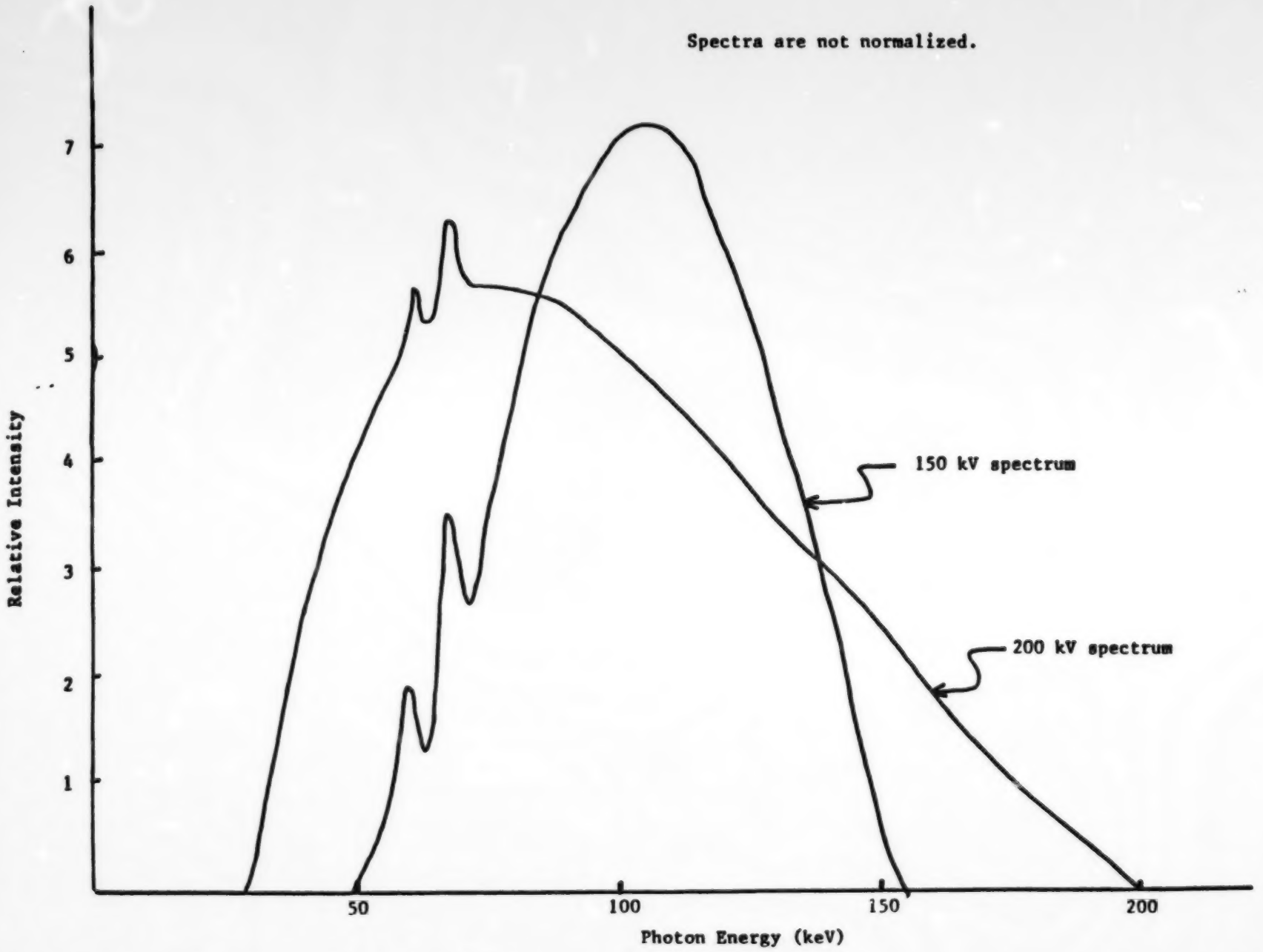

FIGURE 1. Comparison of the 150 and 200 kV photon spectra.

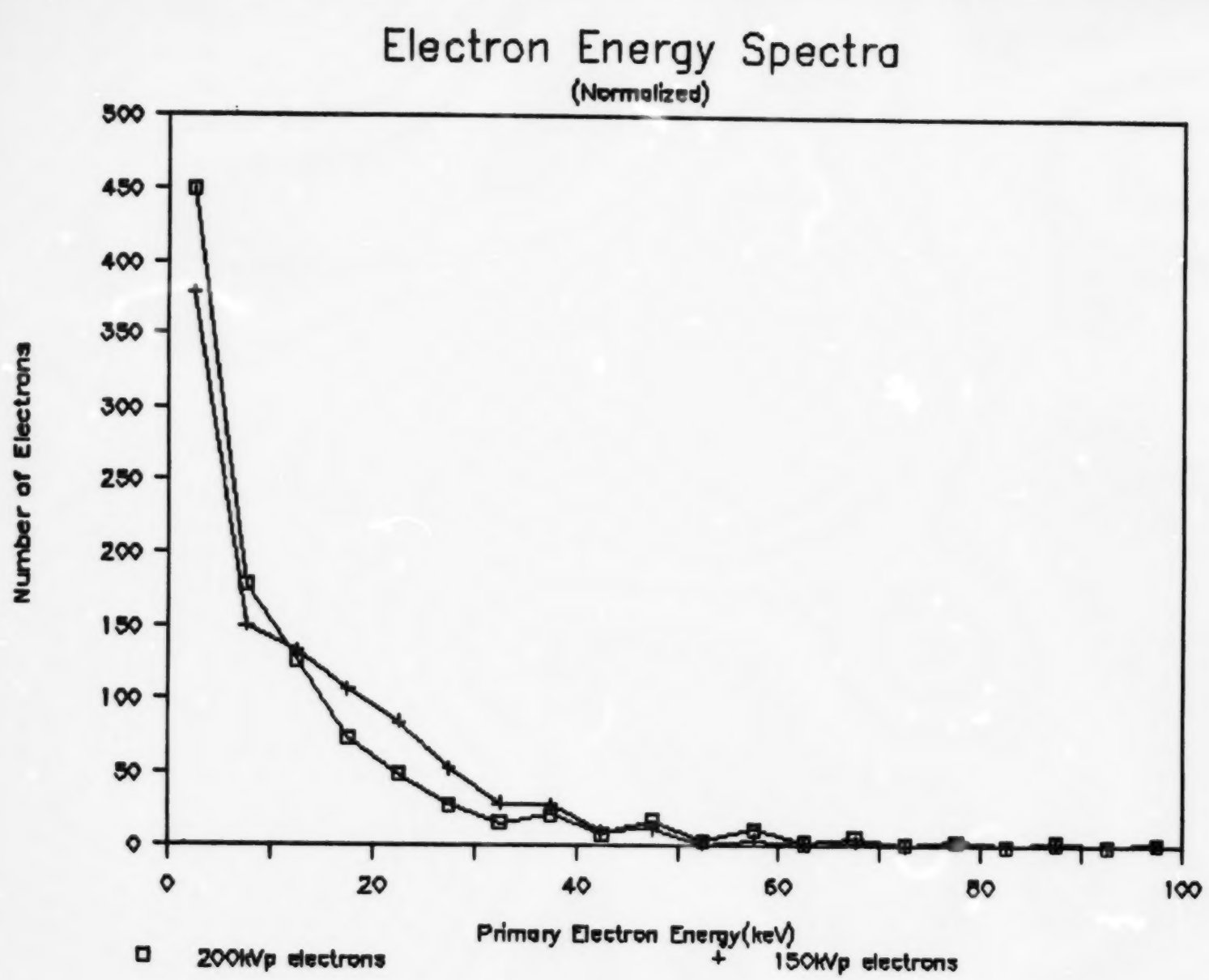

FIGURE 2. Spectra of primary electrons produced in water by the photons from the 150 kV and 200 kV X-ray sets.

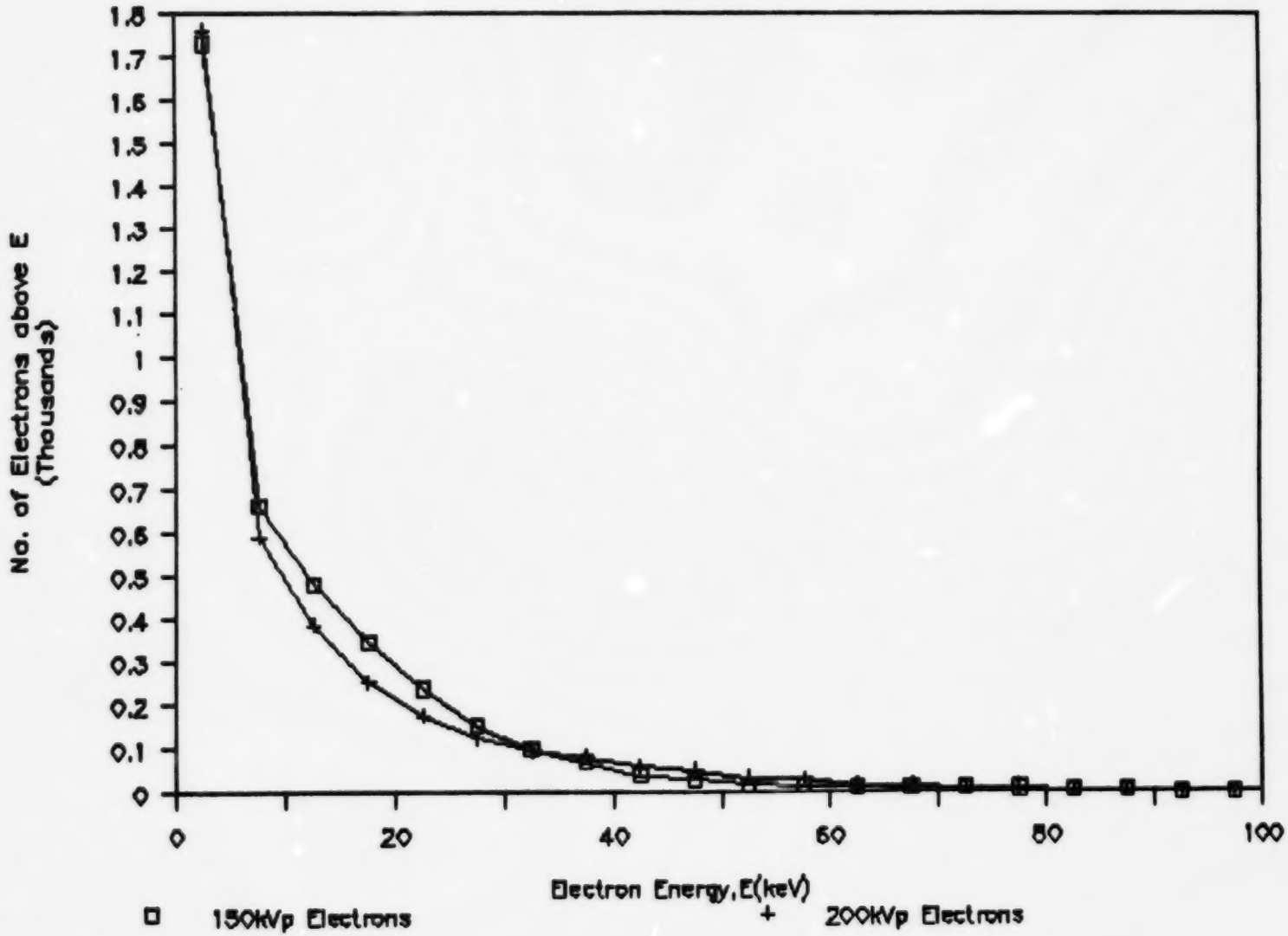

FIGURE 3. The downward cumulative energy distribution of all electras produced in tissue by the 150 kV and 200 kV sets.

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APPENDIX C

POSTMORTEM PROCEDURES

Procedures for Checking and Sampling

1. Colony should be checked daily, using the "inventory method", i.e. animals in each cage must be counted and the number cross-checked for accuracy against the cage tag.

2. If an animal does not look healthy, it should be put separate in a shoebox cage for observation, having appropriately tagged both cage of origin and the shoebox cage. The animal must be weighed daily and the weights recorded.

3. If the animal is moribund and/or suffering, it should be euthanized by decapitation, at which time blood is collected for WBC, RBC and a blood smear. Other tissues are collected as per Pathology Form, indicated by an asterisk. Additional tissues and samples must be taken of organs that look grossly abnormal. A medical history (as much as possible) and any other gross observations (e.g. anemia, jaundice, texture and colour of organs, etc.) should be recorded and may prove very useful in diagnosis.

4. If an animal is found dead in its cage, then describe where and under what circumstances the animal is found. Record when death in your opinion took place. Always collect tissues as per Pathology Form, irregardless of time of death. After collection of tissues, put animal in a platic bag with the same number as the Sample number and put it in the freezer. In this way we can have another look at the carcass.

5. Tissue collection procedure:

   (a) Preparation:
   - sample bottle filled with formalin (10% buffered neutral formalin from Fisher Scientific)
   - fill in "History and Miscellaneous Information" on the pathology form
   - record animal's date of death and sample number on the cage card
   - have ready a "mini-blood collection vial".

   (b) Carcass condition - record how long in your opinion this animal has been dead (if found dead in cage).

   (c) If animal was alive, then animal should be anesthetized (bell jar with methoxyflurane) and decapitated, followed by immediate blood collection in the "mini-blood collection vials". Make sure to "flick" the vial gently with your finger during collection, and also to keep the collection time to less than 30 seconds. These precautions are essential so that no clots form in the blood which will dramatically change the WBC and the RBC. If a clot can be seen then record this.

(d) Take the blood down to the hospital and using the Coulter Counter obtain a value for WBC and RBC. Enter these values on the Pathology record. Also mark a slide with the sample number and do a bloodsmear which is air dried immediately and must be stored in the box marked "blood" in the fridge of the Animal Facility.

(e) Open the skin on the ventral (lower) side of the animal across the length of the animal. Look for any abnormalities in the subcutaneous tissues including the lymph nodes under the front and hind legs. If these lymph nodes are large, then take one sample.

(f) Open the abdominal musculature across the entire abdomen, and cut through the ribs on one side of and close to the sternum all the way along the sternum. Nov cut along the other side of the sternum and remove it from the animal. Trim off excess muscle and lay it on a hard surface. Using a scalpel blade "slit" across each segment of the sternum so that the formalin can reach the marrov. Drop the sternum in the sample bottle.

(g) Look for any gross abnormalities in any of the internal tissues and take samples as appropriate, recording any particulars on the Pathology Form.

(h) In the absence of any gross abnormalities take a small sample of the liver, both kidneys and the whole of both adrenal glands. The spleen should be removed and cut transverse in the middle.

(i) At this point the "pluck" should be removed. This is the combination of the heart, lungs, thymus, which are all attached to one another at the mediastinum, through which the esophagus goes. To remove the pluck, dissect away the tissues which attach it to the back of the animal, starting at the lungs, lifting it up as you go and continuing towards the head. Once the lung and heart are loose from the back of the animal, you can cut through the esophagus and take it out of the animal. With a 1 cc syringe and 27 gauge needle, inject some formalin into each lobe of the lung and into the heart. Then drop the whole pluck into the sample bottle.

(j) Also we would like to collect approximatley two vertebrae. To do this use a bone rongeurs and sever the spinal cord in two places which are about 2-3 vertebrae apart. Lift the segment out, trim off the excess muscle and use a scalpel blade to slit the vertebrae so that the formalin will be able to fix the marrov.

PATHOLOGY RECORD

ANIMAL IDENTIFICATION

Sample No:	HISTORY and MISCELLANEOUS INFORMATION
Treatment:	
DOB:	
Set:	
Cage:	
Animal:	
Disposition: D=dead, E=euth, A=acc. m=morib, S=suffering	
Date:	

GROSS FINDINGS Necropsy by:_____ Histology ___ taken
___ missing

Carcass Condition	*	
Blood	*	Smear:_____
Lym. Nd (Cerv.) (Inguin.) (Axill.)(Mesent.)		
Marrow (Sternum) (vertebrae)	* *	
Pituitary		
Lung	*	
Heart		
Thymus	*	
Liver	*	
Gall Bladder		
Spleen	*	Smear _____
Kidney	*	
Adrenal Gland	*	
Gastrointestinal		
Bladder		
Anus		
Testis/Ovary		
Sem.Ves./Uterus		
Ext. Genitalia		
Skin/Mouth/Lips/Eyes		